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ORIGINAL ARTICLE

Presence of environmental coagulase-positive staphylococci, their clonal relationship, resistance factors and ability to form biofilm

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KEYWORDS

Multidrug-resistant;
Biofilm;
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Environmental

Abstract Coagulase-positive staphylococci (CoPS) are opportunistic pathogens carrying various mechanisms of resistance that have a large number of virulence factors, and whose ability to induce illness is associated with the host. This study aimed to investigate the presence of environmental coagulase-positive staphylococci, their susceptibility profile, clonal relationship and ability to form biofilm. The 16S rRNA genes from CoPS isolates were analyzed, and their antibiotic susceptibility was evaluated using the agar dilution method in accordance with Clinical and Laboratory Standards Institute guidelines. The clonal profile was obtained by pulsed-field gel electrophoresis (PFGE) and biofilm formation was measured by a crystal violet retention assay. A total of 72 *Staphylococcus* spp. strains were isolated from air, metal surfaces, and nostrils from humans, dogs, cats, and birds. Three species were identified: *Staphylococcus aureus* (17%), *Staphylococcus intermedius* (63%), and *Staphylococcus pseudintermedius* (21%). Ninety three percent (93%) of the strains were resistant to at least one of 13 tested antibiotics. *S. pseudintermedius* strains were the only resistant ones to methicillin while most of these isolates were multidrug-resistant, had significantly higher ability to form biofilm and PFGE grouped into seven different patterns, without showing clonal dispersion among animals and environmental isolates. This study suggests that dogs, cat, and air are environmental sources potentially carrying multidrug-resistant *S. pseudintermedius*, which survives in different environments through biofilm formation and

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PALABRAS CLAVE

Resistente a múltiples antibióticos;
Biopelícula;
Electroforesis en gel de campo pulsado;
Estafilococos coagulasa-positiva;
Ambiental

multidrug resistance, characteristics that can be transmitted horizontally to other bacteria and exacerbate the problem of antibiotic resistance in humans.

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Presencia de estafilococos coagulasa positiva ambientales, su relación clonal, factores de resistencia y habilidad para formar biopelícula

Resumen Los estafilococos coagulasa-positiva (CoPS) son patógenos oportunistas, portan varios mecanismos de resistencia, tienen un gran número de factores de virulencia y su capacidad para inducir la enfermedad está asociada con el hospedero. El objetivo de este estudio fue investigar la presencia de CoPS en el medio ambiente, su perfil de sensibilidad a los antibióticos, su relación clonal y su capacidad para formar biopelícula. De los aislamientos de CoPS se analizaron los genes 16S ARNr y se evaluó la sensibilidad a los antibióticos mediante el método de dilución en agar según el CLSI. El perfil clonal se obtuvo por electroforesis en gel de campo pulsado (PFGE) y la formación de biopelícula se analizó por retención de cristal violeta. Se aislaron 72 cepas de *Staphylococcus* spp. a partir de aire, superficies metálicas y narinas de humanos, perros, gatos y aves. Se identificaron tres especies: *Staphylococcus aureus* (17%), *Staphylococcus intermedius* (62%) y *Staphylococcus pseudintermedius* (21%). El 93% de las cepas fueron resistentes al menos a uno de 13 antibióticos probados. Los aislamientos de *S. pseudintermedius* fueron los únicos resistentes a meticilina y la mayoría fueron resistentes a múltiples fármacos, tuvieron una capacidad significativamente mayor para producir biopelícula y la PFGE los agrupó en 7 diferentes patrones, sin mostrar dispersión clonal entre los aislamientos de animales y de medio ambiente. Este estudio sugiere que los perros, los gatos y el aire son fuentes ambientales potencialmente portadoras de *S. pseudintermedius* resistente a múltiples antibióticos. Este agente sobrevive en diferentes entornos en virtud de la formación de biopelículas y la resistencia a múltiples antibióticos, características que pueden transmitirse horizontalmente a otras bacterias y, por ende, exacerbar el problema de la resistencia a los antibióticos en humanos.

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Introduction

Staphylococci are catalase-positive Gram-positive cocci. The different species can be distinguished by their ability to ferment sugars and produce coagulase. Seven species of coagulase-positive staphylococci (CoPS) have been identified: *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus schleiferi* subsp. *coagulans*, *Staphylococcus hyicus*, *Staphylococcus lutrae*, *Staphylococcus delphini* and *Staphylococcus pseudintermedius*⁷. CoPS commonly colonize the skin and mucous membranes; furthermore, staphylococci have the ability to survive in almost any environment. CoPS are opportunistic pathogens; they have a large number of virulence factors, and their ability to induce illness is usually associated with the host. CoPS carry various mechanisms of resistance. Particularly, *S. aureus* became methicillin-resistant by acquiring a genomic island of resistance known as chromosomal cassette mec (SCCmec I-VII), and is a variable genetic element. The island is present constitutively in the *orfX* gene, and depending on the type, has a specific recombinase *ccr*, which allows to carry other resistance genes harbored in small plasmids or transposons¹².

The epidemiology of staphylococci has changed in recent years, as they can cause nosocomial and community infections, and the importance of *S. aureus* has increased because it can cause many pathological conditions ranging from simple skin infections to invasive processes such as pneumonia and osteomyelitis. Moreover, *Staphylococcus epidermidis* is considered a harmless commensal bacterium of the human skin, even an accidental pathogen¹⁶. However, at present, this bacterium is recognized as an important human pathogen and is one of the main causes associated with medical devices such as peripheral or central intravenous catheter-related infections. It also causes keratitis and endophthalmitis, contamination of contact lenses, urinary catheter infections, bacteremia, mediastinitis and other infections. Both species are reported to have high rates of resistance to methicillin, and there is an increasing number of reports on their reduced susceptibility to vancomycin^{5,20,21}.

It is known that staphylococcal species exhibit host specificity, and the species of clinical CoPS specimens differ from those isolated from animals, which also differ among host species. For example, the predominant species in ruminants,

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